BRUCELLOSIS

Dr. Ravi Sikrodia
Assistant Professor
Department of Veterinary Microbiology
College of Veterinary Science and A.H., Mhow
Introduction

1. Brucellosis is considered as one of the most wide spread zoonosis in the world.

2. The importance of this contagious disease is the economic impact on livestock industry.

3. Causes severe hazard to human health, through either direct contact with infected animals or the consumption of contaminated milk and dairy products.
Brucellosis: Synonyms

**Human Disease**
- Malta Fever
- Undulant Fever
- Mediterranean Fever
- Rock Fever of Gibraltar
- Gastric Fever

**Animal Disease**
- Bang’s Disease
- Enzootic Abortion
- Epizootic Abortion
- Slinking of Calves
- Contagious Abortion
- Ram epididymitis
**History**

**Sir David Bruce (1855-1931)**

- British Army physician and microbiologist who discovered *Micrococcus melitensis*

Nomenclature today is credited to **Sir David Bruce**

*Brucella abortus* and *Brucella melitensis*

**Bernhard Bang (1848-1932)**

- Danish physician and veterinarian Discovered *Bacterium abortus* could infect cattle, horses, sheep and goats
Brucella Organism

- *B. abortus* (cattle)
- *B. melitensis* (sheep & goats)
- *B. ovis* (sheep)
- *B. suis* (pigs)
- *B. canis* (dogs)
- *B. neotomae* (wood rat)
# Brucella species:

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Species</th>
<th>Biovar</th>
<th>Natural host</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Brucella abortus</em></td>
<td>1-9</td>
<td>Cattle</td>
</tr>
<tr>
<td>2.</td>
<td><em>Brucella melitensis</em></td>
<td>1-3</td>
<td>Sheep and Goat</td>
</tr>
<tr>
<td>3.</td>
<td><em>Brucella ovis</em></td>
<td>1</td>
<td>Sheep (Ram)</td>
</tr>
<tr>
<td>4.</td>
<td><em>Brucella suis</em></td>
<td>1-5</td>
<td>Swine</td>
</tr>
<tr>
<td>5.</td>
<td><em>Brucella canis</em></td>
<td>1</td>
<td>Dog</td>
</tr>
<tr>
<td>6.</td>
<td><em>Brucella neotomae</em></td>
<td>1</td>
<td>Wood rat</td>
</tr>
</tbody>
</table>
Organisms

- Brucella are small (0.4-0.8 × 0.5-1.5μm), non-motile, non-capasulate, **gram-negative** coccobacilli.

- The organism is **aerobic to microaerophilic**.

- Intracellular

- All strains grow best in a medium enrich with animal **serum** and **glucose**
Biochemistry

- Oxidase $+ve$
- Nonfermentative
- Urease $+ve$ \ catalase $+ve$
- $H_2S$ produced by *B. abortus* and *B. suis*
**Antigenic Structure and Classification**

- Two main antigen: **A** and **M**
- The three main Brucella differ from one another in the amount of the two main antigen they have in common:
  - *B. abortus*: **A**:M = 20:1
  - *B. melitensis*: **A**:M = 1:20
  - *B. suis*: **A**:M = 2:1

The difference in ratio between A and M Antigens.
The designation of the antigens in cultures composed of smooth and rough colonies

<table>
<thead>
<tr>
<th>NO.</th>
<th>Species</th>
<th>Type of colony</th>
<th>Type of surface antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Brucella abortus</em></td>
<td>Smooth</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>m</td>
</tr>
<tr>
<td>2.</td>
<td><em>Brucella melitensis</em></td>
<td>Smooth</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M</td>
</tr>
<tr>
<td>3.</td>
<td><em>Brucella suis</em></td>
<td>Smooth</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>m</td>
</tr>
<tr>
<td>4.</td>
<td><em>Brucella ovis</em></td>
<td>Rough</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>5.</td>
<td><em>Brucella canis</em></td>
<td>Rough</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>---</td>
</tr>
</tbody>
</table>

- The Production of **monospecific antisera** to A and M antigen can be used in the identification of the *Brucella* species.
- *Br.canis* and *Br.ovis* grow as **rough colonies** that possess R antigen.
RESISTANCE

- The organism killed at 60°C
- IN milk = killed by the pasteurization
- In foetus = for several month
- Organism susceptible to the sunlight, Acidic pH, disinfectant.

Can survive in soil for 70 days and for about 35 days in water.
WHO ARE AT RISK?

- Cattle ranchers/ dairy /pig/sheep and goat farmers
- Veterinarians
- Abattoir workers
- Meat inspectors & Lab workers
- Consumers of unpasteurized dairy products
TRANSMISSION IN ANIMALS

• Ingestion of infected tissues or body fluids, contaminated feed or water, or after licking an infected placenta, foetus or genitalia of another cow, after it has aborted.

• Infected bulls may excrete the organism in their semen.

• Congenital transmission may occur through in utero infection.

• Contact with infected tissues or body fluids: Mucous membranes, injections.

• Venereal: Swine, sheep, goats, dogs and other species.
TRANSMISSION TO HUMANS

- **Conjunctiva** or **broken skin** contacting infected tissues
  - Blood, urine, vaginal discharges, aborted fetuses, placentas

- **Ingestion**
  - Raw milk & unpasteurized dairy products
  - Rarely through undercooked meat

- **Inhalation** of infectious aerosols
CULTURAL CHARACTERISTICS

- Brucella colonies are visible after **3-5 days** incubation period at 37°C.

- They are **aerobic** or **microaerophilic**.

- Brucella colonies are 1-2 mm in diameter, round, entire, smooth, glistening, translucent, and a pale honey color when plates are viewed in the daylight.

- All strains grow best in a medium enrich with **animal serum** and **glucose**

- Require **5-10% carbon dioxide** (except *B. melitensis*)
Culture media:

There are two major types of media for cultivation of Brucella:

**A. Basal Medium**
- **commercial media**, e.g. Brucella medium base, Trypticase soy agar, Columbia agar, Serum-dextrose agar or Glycerol-dextrose agar.
- The addition of 2-5% **Bovine or Equine serum** is necessary for the growth of strains such as *B. abortus*.

**B. Selective Media**
- Most widely used medium is **Farrell’s medium**, prepared by the addition of six antibiotics:
  - Polymyxin B sulphate, Bacitracin, Cycloheximide, Nalidixic acid, Nystatin, Vancomycin
  - Skirrow agar for Brucella and Campylobacter
Why Brucella mainly affects genital and mammary tissues?

- Erythritol, a polyhydric alcohol which acts as a growth factor for brucellae, is present in high concentrations in the placenta of cattle, sheep, goats and pigs. (Thus, the brucella organisms are having affinity for gravid uterus.)
- Also present in mammary gland and epididymis.
- In chronic cases organisms localize in joints or intervertebral disc.
**BRUCELLOSIS: ANIMAL DISEASE**

- Chronic infection *Brucella*

- **Infect organs rich in** erythritol **like udder, uterus, placenta and epididymis**

- **Clinical Signs:** abortion and sterility

- **Asymptomatic carrier:** Shed large numbers of organisms in milk, urine, and reproductive tissues
**B. abortus** infection: in cattle

- **Third trimester** abortions with *B. abortus*

- Retained placenta
  - Once expelled will have a **leathery appearance**

- Birth of dead or weak calves
  - Respiratory distress and lung infections

- Low milk yield Will shed organisms in milk for life

- In male-orchitis & epididymitis
**B. melitensis causing:** Sheep and Goat

- *B. melitensis* causing **late term abortions**
  - Retained placenta
  - Birth of dead or weak lambs/kids

- Goats - articular and periarticular **hygroma** localizations

- *B. ovis* causing abortions, fertility problems
  - **Orchitis, epididymitis**
  - Abnormal breeding soundness
  - Organisms present in semen
B. suis: Swine

- Prolonged bacteremia
- Abortion, early or late gestation
- Fertility problems
  - Sows temporary
  - Boars- unilateral or bilateral orchitis
- Lameness, posterior paralysis, spondylitis, metritis, abscesses
**B. canis**: Dogs

- Susceptible to
  - *B. melitensis, B. abortus, B. canis* and *B. suis*

- *B. canis* causes abortions
  - **Last trimester** of pregnancy
  - Prolonged vaginal discharge
  - **Bacteremia** for up to 18 months post-exposure
  - Failure to conceive, stillbirths, prostatitis, epididymitis
**Diagnosis**

- Direct microscopic examination
- Isolation of organism
  - Blood, semen, other tissues
- Serology
- Brucella milk ring test
- Biological test
- Demonstration by fluorescent antibody of organism in clinical specimen
  - Placenta, fetus
Direct microscopic examination:

- Prepare the impression smear (samples like cotyledons, aborted fetal stomach content or spleen, uterine discharge)

- Perform gram’s staining staining

- Examine under microscope

- On examination they may be found in neutrophils or macrophages

- **Koster’s staining:** To demonstrate brucella in smear from placenta
Isolation

- Most preferred samples: cotyledons, aborted fetal stomach content or spleen, uterine discharge, milk and semen
- Inoculate the sample on dextrose agar or blood agar, with 5-10% CO2 for 3-6 days.

Brucella colonies on BA
The most common serological test

1. Rose Bengal Test (RBT).
2. Milk Ring Test (MRT) /Herd test.
4. Enzyme Linked Immuno Sorbent Assay (ELISA).
A. **Principle**
   The buffered – acid antigen stained with Rose Bengal is used for the early detection of Brucella specific agglutinins.

B. **Reagents**
   Rose Bengal Antigen

**Result:**
- **No agglutination** = Negative result.
- **Visible agglutination** (even slight) = Positive results
**Milk Ring Test (MRT)**

The Ring test method is used for the detection of brucella antibody in milk samples. This technique is very easy to perform it especially in dairy herds.

A. Principle

It is uses the principle of **agglutination** between antibodies contained in milk and dyed colored bacterial antigen of brucella to form antigen-antibody complexes that are progressively carried by the fat towards the surface of the milk and formed a blue violet ring.

B. Reagents

- Inactivated bacterial culture of *Brucella abortus S 99* stained with hematoxyline.
Procedure

1. Carefully mix the tested milk, and put 1 ml in a test tube.
2. Add 50 µl of the antigen and mix carefully.
3. Place in incubator for 1 hour at 37 °C, after that for (18-20 hours) at 4 °C, and then read the result.

Results

1. Ring of cream equal or more colored than the underlying milk = Positive result.
2. Ring of cream less colored than the underlying milk = Negative result.
STAT: Standard Tube Agglutination test

- Quantitative test
- Titre is calculated on the basis of this test which is used to cull or slaughter the animals
- In this Colourless Brucella antigen is mixed with different dilution of serum, to quantitate the antibodies present in the suspected serum of animals.
DIFFERENT CHARACTERS TO IDENTIFY THE SPECIES

<table>
<thead>
<tr>
<th>species</th>
<th>CO2 incubation</th>
<th>H2S production</th>
<th>Urea hydrolysis</th>
<th>Growth in Carbal/basic fuschin</th>
<th>Growth in Thionine</th>
<th>Growth in Malachite green</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. abortus</td>
<td>required</td>
<td>2-4 days</td>
<td>Very slow</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>B. suis</td>
<td>Not required</td>
<td>7 days</td>
<td>rapidly</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>B. melitensis</td>
<td>Not required</td>
<td>1 day</td>
<td>slowly</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- **Dye inhibition test**: The species of brucella have differences in ability to grow in medium containing basic fuschin, thionine and malachite green
- **RTD(Routine test dilution)**: Tbilisi phage
Thank You